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THE AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

MICROORGANISMS OF THE SOIL[1]

SUCH statements as "the soil is not a mere sponge, but is teeming with life" or that "the earth is one of nature's vast laboratories in which microscopical wonder-workers perform incredible experiments" may have been unusual enough at one time to attract attention; but no longer is the presence or performances of these inhabitants of the soil of such novelty as to startle or dismay us. Indeed so accustomed have we become to the idea that each gram of the upper layers of the earth is filled with its millions or billions of bacteria, that the tendency is to ascribe all functions of the soil to its micro-flora and no theory is too bizarre, no miracle too improbable, so long as we may fall back upon the soil bacteria to account for it.

The apologetic statement only a short while ago of a German investigator, that perhaps, after all, the chemical condition of the soil might have almost as much to do with a given condition as the bacteria, illustrates, if nothing else, the great changes that have taken place in our conception of the constitution of the earth's surface during the last twenty years. To attempt to indicate the relative places of the various aspects of science which are concerned in problems of the soil would be as useless as it is impossible, but I might as well say at once that I do not feel that the situation calls for any particular glorification of the biologist. I do not wish to

[1] Presented at the Symposium on Soils at the Washington meeting of the American Association for the Advancement of Science.

be misunderstood as minimizing the importance of a real knowledge of the activities of the micro-flora and fauna of the earth. Rather would I hope to emphasize this aspect of the question, which I suppose is what was expected when the subject was assigned to me. It is quite as important, however, to point out the unsatisfactory state of the investigations at present and the futility of generalizing from a few known facts, acquired by disregarding a vast number of unknown, but nevertheless real, factors.

Fischer, you will remember, after a more or less critical review of the situation, came to the conclusion that we do not now possess a method of bacteriological examination of soils, which is of the least practical value. While not subscribing to this view, it must be confessed that a study of the literature on the subject indicates that much of fundamental importance remains to be done before we can hope that an investigation of the microorganisms of the soil will result in really solving some of the perplexing problems of fertility now confronting us. Even the nomenclature of the subject is so indefinite at the present time, that within the past year we have had conflicting uses of such familiar terms as "nitrification" and "nitrogen fixing" and there certainly is need for some such unification and strict definition of terms as that suggested by Lipman.

An enumeration of all the methods now in vogue for the bacteriological examination of the soil would show that the technical side of the subject is in much the same condition that water bacteriology was fifteen years ago, and until there is more uniformity in methods employed, by which comparative tests can be made, we shall not gain much from the results of the steadily increasing number of workers in this field. It is true that new points of view are occasionally presented and a distinct step in advance has been the emphasis recently placed upon the study of the organism, as far as possible, in its natural environments, rather than in artificial solutions. The work of Vogel, Stevens and others, has done something to make possible an agreement in parallel laboratory and field experiments; but, after all, progress in this line has been chiefly through pointing out the errors of others and has not resulted, as yet, in the formulation of a standard. We are much farther along in knowing what not to do, but like the Sherman law, the situation calls for some affirmative legislation.

There is also considerable evidence that we have been so obsessed by the pure culture idea, that conclusions drawn from experiments performed under such conditions are entirely unwarranted. If it is true that mixed cultures of *Azotobacter chroococcum* and *Pseudomonas radicicola* will fix almost twice as much nitrogen as either alone, to say nothing of the necessary interaction between various groups in making available green manures, phosphoric acid, lime nitrogen, etc., it is very evident that conclusions drawn from the study of a single organism can not be applied to the conditions actually existing in the soil. We might as well assume that a superior being, dipping down into our atmosphere and selecting a single individual, would be able to arrive at the various functions and activities of man on the face of the globe, by observing his behavior under such artificial conditions as it would be possible to maintain in a heavenly laboratory. It is neither necessary, nor advisable, of course, that we abandon the pure culture method. But we should recognize the limitations of our present technique and cease to generalize from such inadequate data.

Not only are standardization of methods and interpretation of results, as well as a still further recognition of the effect of various groups of bacteria, one upon the other, much to be desired; but an appreciation of the fact that something else than the bacteria go to make up the microscopical life of the soil, must be more generally taken into consideration, in our attempts to find out what actually goes on in the ground. It must be confessed that thus far any knowledge of the algal, fungal or protozoal inhabitants of the soil has tended to confuse rather than clarify any conclusions regarding the phenomena induced by a single group of organisms.

Perhaps no better example of the chaotic conditions of the present status of the microbiology of the soil can be cited than in the recent revival of a consideration of the effect of heat and various so-called antiseptics on crop production, and the supposed relations of protozoa to the problem. That the addition to soil of carbon bisulphide, toluol, ether and similar agents, will *under certain conditions* benefit *some* crops, has of course been known for nearly twenty years, and as early as 1888 Frank believed that sterilizing soil with steam increased the solubility, or availability, of mineral and organic substances.

Various theories, from the mere removal of superabundant, though harmless, bacteria, to the destruction of toxins, have been proposed to account for this beneficial effect, but it remained for Russell and Hutchinson, of the Rothamsted Station, to stimulate interest in the subject. These investigators, in October, 1909, announced that they had found the increased productiveness of partially sterilized soil to be due to an excess of ammonia, arising as a result of the bacterial decomposition of soil substances, these bacteria being able to multiply enormously on account of the removal, by heat or volatile substances, of large protozoa which normally feed upon the bacteria. This announcement was hailed both in this country and abroad as the greatest discovery pertaining to the soil, since Hellriegel's interpretation of the beneficial effect of bacteria in the root-nodules of legumes!

A student in my laboratory becoming interested in the problem, undertook a considerable number of preliminary experiments, the results of which seemed to warrant a more elaborate investigation into the effect of soil sterilization upon crop production. It is not necessary to go into details at this time, but laboratory, greenhouse and field tests all indicated most decidedly that the theory of Russell and Hutchinson is *not* of universal application, and the importance of the protozoa, so far as their effect upon bacteria is concerned, has been overestimated. It is true that Russell and Hutchinson themselves considered the removal of the protozoa as being but one factor concerned in the benefits accruing to plants, by the use of antiseptics, and it may be that the prominence given to this aspect of their work is due to the advertising propensities of those not immediately concerned with the investigations. This is unfortunately sometimes the case. The fact remains, however, that in many of the comments published by those rather closely associated with Russell and Hutchinson, the effect of antiseptics upon protozoa is deemed to be the only one worth considering, and to which all resulting benefit is due.

Within the last few months, several papers have appeared which likewise fail to agree with Russell's and Hutchinson's results. Goodey, publishing in the *Proceedings of the Royal Society*, shows that at least one important group of protozoa, micro-

photographs of which have been used most extensively to illustrate popular articles on the subjects, can have no part whatever in disposing of beneficial bacteria or influencing in any appreciable way the fertility of the soil. For it is found that the ciliated protozoa which are so characteristic a feature of cultures made from soil, exist only in the encysted stage in natural soil conditions. There remain, of course, the amœbæ and flagellates, about the condition of which in soil we are not certain as yet. But the fact that all of these organisms are able, within a short time after being removed by disinfectants, to reestablish themselves in soils, would seem to indicate that even though they might have some direct effect upon the bacterial content of the soil, the removal is so transient that the effect on crop production is negligible. This is no place for figures, but if it were I could show as the result of tests, extending over a wide field, that the number of protozoa, including flagellates, ciliates and rhizopods, existing in the soil three days after treatment with various percents of toluol, carbon bisulphide, etc., may equal or even exceed the number originally present.

Koch and Fred at the Agricultural Institute of the University of Göttingen, since the appearance of Goodey's paper, have each published independently upon the effect of ether and carbon bisulphide on lower and higher plants and conclude that for both the micro-flora of the soil and the crop it bears, the beneficial effect is purely stimulative—simply the old idea of all poisons being beneficial to growth if sufficiently dilute.

Greig-Smith, in spite of the apparent refutation of the toxin theory of Russell and Hutchinson, returns to it as affording the best explanation of the observed results. He claims to have extracted from soil a substance which is filterable through porcelain and which is toxic to bacteria. This toxin thus retards the growth of higher plants by the destruction of beneficial bacteria, but in turn is destroyed by the application of heat or volatile antiseptics. An additional effect of these agents is upon the so-called "agricere," which, according to Greig-Smith, is a mixture of saponifiable and unsaponifiable bodies, coating or waterproofing particles of soil. When heat or certain wax solvents are added to the soil, they alter the distribution of the earth wax, carrying it to the surface and causing it to segregate on the points of the soil particles. The beneficial effect of the removal of the waterproof covering is of course that the constituents of the soil are more easily attacked by the bacteria and rendered available for plant nutrition.

Bottomly, before the British Association this summer, confirmed to a certain extent the work of Greig-Smith by demonstrating the injurious effect of the "bacteriotoxines" upon the germination of seeds and their subsequent growth, the harmfulness of which could be prevented by first heating the soil.

Without further reference to contradictory results obtained by various investigators since the announcement of Russell and Hutchinson, experiments in my own laboratory indicate that the matter is probably incapable of being satisfactorily explained by any of the single factors which have been suggested. The one fact which does seem to be fairly well established is that the temporary removal from the soil of the protozoa has but little bearing on the problem. Neither is it by any means certain that the use of heat or antiseptics is universally favorable to all crops on all kinds of soil and it seems probable that the character of the soil, as well as the kind of crop, will have to be taken into considera-

tion before we can have a true explanation of why some crops are benefited by the so-called sterilization of some soils.

Aside from a very few pathogenic forms, but little is specifically known of the fungus flora of the earth. That fungi may be as abundant as the bacteria, particularly in uncultivated soils and that below the humus-containing layer, they may considerably exceed the bacteria, has been ascertained by a few analyses. But what they do and how they do it, is for the most part a matter of conjecture. To those familiar with the rapidity and certainty with which some of the higher fungi reduce organic to inorganic matter, it is evident that there is no group of organisms present in the soil that would seem to be more capable of producing profound changes in its environment. Not only do we know that a considerable amount of the decay in animal and vegetable tissue, particularly the early stages, is due to the higher fungi, but the work of Czapek and Kohn, showing that *Penicillium* and *Aspergillus* when supplied with ammonium chloride set free hydrochloric acid, as well as the demonstration of the production of an organic acid in *Penicillium* by Alsberg and Black, points to still further possibilities by plants belonging to the same, or closely related groups.

Formerly it was supposed that the number of plants whose roots entered into combination with some fungus—presumably for mutual benefit—was limited and confined to but few families. Now it is estimated that about one half of the seed plants possess within their roots some mycorhizal organism and in many notable instances the plant is unable to thrive under natural conditions without its particular fungus. Furthermore, as has been pointed out by Coville, the acidity factor in the distribution of some plants is mycological rather than purely chemical.

Our knowledge of the effect of soil fungi upon the germination of seed is also being extended. Barnard has recently shown that seed of both the common potato and *Solanum dulcamara* fail to germinate in the absence of their mycorhizal parasite, while 40–90 per cent. begin to grow in the presence of this fungus.

Whether the considerable number of wild yeast-like organisms occurring abundantly in many soils, are capable of producing profound changes in their habitat is still problematical. That certain of these may fix atmospheric nitrogen in the laboratory seems to have been demonstrated, and it appears reasonable that should conditions in the earth be favorable, we might expect yeasts to have a decided effect either upon the soil, or its inhabitants. Despite the necessity of yeasts having secondary breeding places, such as aqueous extracts from fruits and other vegetable matter, the soil must be considered the chief abode of these fungi, and not only during the colder months, but throughout the entire year.

Even less is known about the algal content of the soil than of its fungus constituents. The older literature is full of references to the nitrogen-fixing power of both grass-greens and blue-greens, but it is a striking fact that since the introduction of the pure culture method for algæ, there has been no authenticated demonstration of the power of these plants to add in the slightest degree to our store of fixed nitrogen. It is true that Heinze working with impure cultures of *Nostoc* thought he had demonstrated by a process of elimination its ability to fix nitrogen. Since *Azotobacter* was not present and the fungus in the culture could not by itself fix nitrogen, he assumed that the nitrogen accumulated must be due to the alga. But this can hardly be accepted as conclusive. While it it possible that some of the blue-greens

may have this power, it is not likely that they are of much importance and there is need of a most careful investigation of the whole subject, now fortunately under way, before we can be at all certain of what the algæ alone accomplish in the soil.

The possible beneficial relationship between the algæ and the bacteria is quite another question. I believe it is not widely known that quite independent of any surface growth of algæ, there exists in the lower layers of the soil an algal flora which in some localities, at least, is equal, bulk for bulk, to the bacterial flora. Exact quantitative estimates are difficult and in the incomplete state of the work, only approximations can be made, but it is safe to say that under some circumstances the individual algal cells, many times larger, of course, than ordinary bacteria, number between three and four million per gram of soil. For the most part these cells belong either to *Anabœna* or *Nostoc,* and without committing myself at this time to the original observations of Brand, recently confirmed by Miss Spratt, that the heterocyst of *Nostoc* and *Anabœna* gives rise to gonidia-like spores, I may say that heterocysts obtained from the deeper layers of the soil often show the contents divided in precisely the way figured by Brand and Miss Spratt. If it be true that the heterocyst is capable of giving rise to spores, it would account, of course, for the large number of isolated cells found in the soil, and further explain how there may be such an abundant algal flora below the surface, which, be it noted, is totally different, as to genera, from the surface film of algæ.

The observations relative to the fixation of atmospheric nitrogen through the association of algæ and bacteria are somewhat more satisfactory than those dealing with algæ alone. We have some experimental evidence for believing that when certain nitrogen-fixing bacteria are growing with some of the blue-green algæ, the amount of nitrogen exceeds considerably that fixed by the bacteria alone and the benefit of the combination upon growing crops is marked. Thus we have an additional complication in dealing with the vital activities of the soil, for it appears we must not only consider the interrelationships between various groups of bacteria in so-called "mixed culture," but the influence of a considerable algal flora must also be taken into account.

No discussion of the microorganisms of the soil would be complete without some reference to the nodule-forming bacteria of legumes. That the practical application of our knowledge of the effect of these, usually, but not always, beneficial bacteria must be demonstrated in the field, rather than the laboratory, goes without saying. However, it is hard to understand how we may hope to gain much definite information either as to the needs or activities of these bacteria, when conclusions regarding them are drawn exclusively from such an inconstant and uncertain source. That much depends upon the virulence of the particular strain of organism is evident and the use of nitrogen-free media, first suggested in this country and some modification of which has since been widely adopted, both at home and abroad, has resulted in increasing materially the percentage of successful inoculations. Whether the conflicting results obtained by different investigators can be harmonized, in the state of our present knowledge, is doubtful, for the conditions are bound to be so various and the bacteria themselves so sensitive to changed environment, that comparable results will seldom be obtained. Indeed, it may not be impossible that *Pseudomonas radicicola* plays a more important rôle outside of the root nodules

than within it, and instead of attempting to induce the legume organism to form nodules on other crops, we should perhaps be paying more attention to the organism as it exists in the soil, independent of the roots of any plant.[2]

In this connection, however, I may say that I now have under cultivation an organism capable of fixing nitrogen within nodules comparable in every way to those found on the legumes, but growing on a family far removed from the Leguminosæ, namely, the Aristolociaceæ.

Of the importance of the bacterial flora in rendering available, to higher plants, the various necessary mineral constituents of the soil, little need be said. That a large number of organisms are able to influence the potash, lime, magnesia, phosphorus and other minerals of the soil solution is well known. It even appears that calcium salts of various organic acids, frequently formed by plants and occurring in soils, may be oxidized to carbonates by a considerable variety of bacteria, thus conserving the lime supply to the last degree. On the other hand, it may be well to point out that the generally accepted theory regarding the action of the so-called iron-bacteria is probably incorrect. Winogradsky's hypothesis, that the soluble bicarbonate in water or soil was absorbed by the organism and, as a result of cell metabolism, changed into ferric hydroxide, was never proved, even by its author. The analogy between the appearance of iron on the walls of these forms and the oxidation processes of the sulphur and nitrate bacteria seems to have been the chief reason for its promulgation. Molisch has shown that iron is not necessary for the growth of these organisms and later other investigators proved that manganese readily replaced the iron. There seems to be no reason, therefore, for assuming that the deposition is in any way connected directly with the metabolism of the plant. Rather is the relationship similar to that existing in certain algæ and an aquatic ascomycete, recently obtained by me. Klebs showed that *Zygnema* could retain in the gelatinous layer surrounding it, not only iron, but aluminium and chromium compounds. Whether this is due to some peculiarity in the wall, or is a sort of reversed chemotaxis, with the plant attracting the metal, instead of the chemical attracting the plant, remains to be seen. Observations made on the fungus above referred to plainly indicate that it is not necessary to ascribe any respiratory or oxidizing function to the process, and if it be vital it must be something in the nature of what we might be permitted to call "vegetable magnetism."

Without going further into details, I think enough has been said to indicate the diverse character, and yet the close interrelationship, existing in the microbiological content of the soil. While it may not appear to simplify the problem, by admitting that the physiologist, the bacteriologist, the mycologist, the algologist and possibly the protozoölogist, to say nothing of the chemist and physicist, must all coöperate before many fundamental problems

[2] Since writing the above, I have learned that Greig-Smith recently presented a paper before the Linnean Society of New South Wales in which he claims, by means of a special medium, to have determined the number of *Pseudomonas radicicola* per gram of cultivated soil, to be at least three millions. He, apparently from the literature on the subject, and not by actual test, assumes that the number of *Azotobacter* and similar nitrogen-fixing organisms is small and consequently concludes that the foremost place in nitrogen fixation in the soil should be given to *Pseudomonas* and not *Azotobacter.* Indeed he goes so far as to maintain that the number of *Pseudomonas* organisms in the soil affords an indication of its comparative fertility.

involving fertility and plant nutrition are finally solved, I am inclined to think this is the only means whereby we can hope for success. At least the information derived in this way is more apt to bring us to the desired state of knowledge than our present independent attitude. "The sciences gain by mutual support," wrote Pasteur. Certainly it is not by an arrogant assumption to one's self that his particular science is the "be all and end all" of human endeavor, that we shall gain any notion of what is really happening in the soil and what it all means!

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PLANT FOOD IN RELATION TO SOIL FERTILITY[1]

I take it that the only justification for me to review the subject of plant food in relation to soil fertility or crop production is the fact that recent publications from the federal Bureau of Soils have strongly affirmed that there is no necessity of applying plant food in the restoration and maintenance of soil fertility. Two principal questions are raised: First, Does plant food applied increase crop yields in harmony with recognized soil deficiencies and crop requirements? Second, Will the rotation of crops maintain the productive power of the soil by avoiding injury from possible toxic excreta from plant roots? I shall try to present facts and data and exact quotations rather than my own opinions concerning these questions of such fundamental importance in relation to systems of permanent agriculture.

In 1804 DeSaussure, the French scientist, first gave to the world a correct and almost complete statement concerning the sources of the food of plants, including not only the confirmation of Sénébier's discovery of the fixation of carbon in the formation of carbohydrates, but also the evidence of plant requirements for the essential mineral elements secured from the soil.

Sir Humphry Davy and Baron von Liebig did much to popularize this information during the following half century; and they were followed by Lawes and Gilbert, whose extensive and long-continued investigations furnished the needed proof that the soil must furnish nitrogen as well as the mineral elements; and finally, only twenty-five years ago, Hellriegel discovered the symbiotic relationship between legumes and bacteria which gives access to the inexhaustible supply of atmospheric nitrogen for soil enrichment.

Briefly, it might be said that for nearly a century the world of science has accepted and taught, and the world of advanced agricultural methods has practised, the doctrine that soil fertility maintenance and soil enrichment require the restoration or addition of plant food, including particularly phosphorus and nitrogen, which are most likely to become deficient in normal soils, potassium where needed, and sometimes lime or limestone, which always supplies calcium, and magnesium as well if dolomitic limestone be used. Of the other five essential elements, carbon and oxygen are secured from the carbon dioxid of the air, hydrogen from water, and iron from the inexhaustible supply in the soil; while the sulfur brought to the soil in rain and otherwise from the atmospheric supply, resulting from combustion and decomposition of sulfur-bearing materials, supplemented by the soil's supply and by that returned in crop residues, appears to be sufficient to meet the plant requirements and the loss by leaching.

After nearly a century of the increasing

[1] Presented at the Symposium on Soils at the Washington meeting of the American Association for the Advancement of Science.